**I. Status Update:**

To be able to breed new varieties with improved seed composition and yield characteristics, more information regarding the genes that control these traits is needed. Regulation of gene activity levels plays a critical role in determination of desirable traits. In brief, a major part of the reason that two varieties differ in these traits is that the varieties differ in the activity levels of important genes. So, by identifying the genes that control variation in gene activity, it should be possible to identify many of the genes that control variations in seed composition and yield. These genes that control variation in gene activity are known as eQTL. In earlier work we identified six candidate eQTL that are predicted to cause improvements in yield and/or seed composition when overexpressed. We named the genes that potentially correspond to these candidate eQTL “ELP” genes. In a field trial in Saint Paul, transgenic soybean lines carrying extra copies of any one of four of these six ELP genes had seed yields that were 19-66% higher than those of the non-transgenic control line. In addition, transgenic lines carrying extra copies of any one of five of the ELP genes had increases in seed protein or oil contents. The objectives of this proposal are to characterize the mechanisms by which ELP genes affect seed yield and composition, conduct field trials of our currently available ELP lines in different areas of Minnesota and generate new ELP transgenic lines using elite Minnesota varieties.

As part of our current research, we planted seeds from the 16 most promising transgenic lines carrying ELP genes. We also planted non-transgenic Wm82, Minsoy and Archer as controls. For each transgenic line we planted 12 randomized plots in a field in Saint Paul, 10 plots in Waseca and 10 plots in Lamberton. Thus we planted a total of 32 plots per transgenic line, across all three locations within Minnesota. During the summer we collected developing seed samples from all 565 field plots. In the fall we collected mature seed samples from all 565 field plots.

Most recently we have measured yield (weight of mature seeds/plot) and seed composition for each of the 565 mature seed samples. We have been analyzing that data and have found that three of the transgenic lines had statistically significant increases in seed yield. One of those lines, line ELP2-08, is particularly promising as it has now displayed increased yields in three field trials in a row. The increase in seed yield for this line in the 2019 trials was 2.4%, on average.

Seed protein levels were increased in six of the transgenic lines characterized in the 2019 field trials. Increases in seed protein ranged from 0.8 to 2.9% (i.e. from approximately 39.9% in the non-transgenic Wm82 parental line to between 40.2 and 41.1% on a dry weight basis in the transgenic lines). Three of these six lines had no statistically significant alterations in seed oil content whereas the other three lines did exhibit statistically significant decreases in seed oil content. In terms of increased protein content, lines ELP1-08, ELP3-02 and ELP3-08 are particularly promising as they have now exhibited significant increases in seed protein content in three field trials in a row. In addition, ELP1-08 did not have a statistically significant alteration in seed oil content in 2019.

Seed oil content was significantly higher in the ELP1-35A line. Seed oil was up 1.2% in this line. In addition, the ELP3-09 line displays several potentially interesting alterations in seed composition. Seed starch levels were increased by 36% and seed sucrose levels by 11% in this line. Similar increases in seed starch and sucrose levels were also observed for this line in the 2018 field trial.

We are continuing to isolate RNA from some of the 565 samples of developing seeds. We will use those RNA samples to measure transgene expression levels so that we may determine the extent to which differences in transgene expression levels correlate with the observed differences in seed yield and seed composition. This information will allow us to determine the optimal transgene expression levels to achieve desirable seed yields and traits.

**Reporting Start Date:**

 02/01/2020

**Reporting End Date:**

 04/30/2020

**Status Report Submission Date:**

 05/29/2020

Project Objectives

**Objectives:**

**Objective 1: Characterize the mechanisms by which different ELP genes affect seed yield and composition.**

Activity: We are continuing to isolate RNA from some of the 565 samples of developing seeds. We will use those RNA samples to measure transgene expression levels so that we may determine the extent to which differences in transgene expression levels correlate with the observed differences in seed yield and seed composition. This information will allow us to determine the optimal transgene expression levels to achieve desirable seed yields and traits.

**Objective 2: Determine seed yield and composition for select ELP transgenic lines grown in different parts of Minnesota.**

Activity: We have been analyzing seed yield and composition data and have found that three of the transgenic lines had statistically significant increases in seed yield. One of those lines, line ELP2-08, is particularly promising as it has now displayed increased yields in three field trials in a row. The increase in seed yield for this line in the 2019 trials was 2.4%, on average.

Seed protein levels were increased in six of the transgenic lines characterized in the 2019 field trials. Increases in seed protein ranged from 0.8 to 2.9% (i.e. from approximately 39.9% in the non-transgenic Wm82 parental line to between 40.2 and 41.1% on a dry weight basis in the transgenic lines). Three of these six lines had no statistically significant alterations in seed oil content whereas the other three lines did exhibit statistically significant decreases in seed oil content. In terms of increased protein content, lines ELP1-08, ELP3-02 and ELP3-08 are particularly promising as they have now exhibited significant increases in seed protein content in three field trials in a row. In addition, ELP1-08 did not have a statistically significant alteration in seed oil content in 2019.

Seed oil content was significantly higher in the ELP1-35A line. Seed oil was up 1.2% in this line. In addition, the ELP3-09 line displays several potentially interesting alterations in seed composition. Seed starch levels were increased by 36% and seed sucrose levels by 11% in this line. Similar increases in seed starch and sucrose levels were also observed for this line in the 2018 field trial.

**Objective 3: Generate improved ELP gene soybean varieties.**

Activity: None during the current reporting period.

**Achievements:**

We have determined that three of our transgenic lines had increased seed yields in the 2019 field trial. The ELP2-08 line is particularly interesting as it has now displayed increased yields (e.g. 2.4% in 2019) in three field trials in a row. We have also determined that six of our transgenic lines have increases in seed protein levels ranging from 0.8 to 2.9% and that one line exhibited a 1.2% increase in seed oil content. An additional line displays very substantial increases in seed starch (36%) and seed sucrose (11%) levels.

**Challenges:**

None during the current reporting period.

**Publication(s)/Symposium:**

None during the current reporting period.

**Tech Transfer:**

None during the current reporting period